

Amendments to the Claims:

All amendments and cancellations to the claims are made without prejudice or disclaimer.  
This listing of claims replaces all prior versions and listings of claims in the application.

Listing of Claims:

1. (Currently amended) A method of evaluating a test agent for the ability to modulate a parameter of heart function in a mammal, the method comprising:
  - (a) contacting a zebrafish heart with a test agent;
  - (b) obtaining a recording of the zebrafish heart;
  - ~~(b)~~ (c) evaluating a parameter of heart function in the zebrafish heart by analyzing the recording automatically using a computer program; and
  - ~~(e)~~ (d) correlating the effect of the agent on the parameter of heart function in the zebrafish with a predicted effect on heart function in a mammal.
2. (Original) The method of claim 1, further comprising generating a dataset correlating a value for the evaluated parameter with cardiotoxicity or probability of cardiotoxicity of the agent.
3. (Original) The method of claim 1, wherein the parameter of heart function is heart rate.
4. (Currently amended) ~~The method of claim 1,~~ A method of evaluating a test agent for the ability to modulate a parameter of heart function in a mammal, the method comprising:
  - (a) contacting a zebrafish heart with a test agent;
  - (b) evaluating a parameter of heart function in the zebrafish heart; and
  - (c) correlating the effect of the agent on the parameter of heart function in the zebrafish with a predicted effect on heart function in a mammal, wherein the parameter of heart function is ejection fraction, contraction fraction, conduction velocity, or repolarization, ~~or Q-T interval.~~

5. (Original) The method of claim 1, wherein the zebrafish is a wild-type zebrafish larva.

6-7. (Canceled)

8. (Original) The method of claim 1, wherein the test agent causes an arrhythmia in the zebrafish heart.

9. (Original) The method of claim 1, wherein the test agent is administered to the culture media of the zebrafish.

10. (Original) The method of claim 1, wherein the test agent is injected into the zebrafish.

11. (Original) The method of claim 1, wherein the zebrafish is a zebrafish larva.

12. (Original) The method of claim 1, further comprising contacting the zebrafish heart with a second test agent.

13. (Canceled)

14. (Original) The method of claim 1, wherein the method is performed in an array format.

15. (Original) The method of claim 1, further comprising contacting the zebrafish with a dye.

16. (Canceled)

17. (Original) The method of claim 1, wherein the test agent is evaluated in combination with a second test agent.

18. (Original) The method of claim 1, wherein the test agent is a small molecule.
19. (Original) The method of claim 1, wherein the test agent is a protein, DNA or RNA molecule.
20. (Currently amended) A method of determining if a test agent is cardiotoxic in a mammal, the method comprising:
- contacting a developing zebrafish with a test agent;
  - obtaining a recording of the zebrafish;
  - measuring a parameter of heart contractility in the zebrafish by analyzing the recording automatically using a computer program, and
  - identifying a test agent that causes an abnormality in heart contractility in the zebrafish as a cardiotoxic agent in a mammal.
21. (Original) The method of claim 20, wherein the parameter of heart contractility is heart rate or QT interval.
22. (Original) The method of claim 20, wherein the abnormality is arrhythmia.
23. (Original) The method of claim 20, further comprising generating a dataset correlating a value for the parameter of heart contractility with cardiotoxicity or probability of cardiotoxicity of the agent.
24. (Original) The method of claim 20, wherein the zebrafish is a wild-type zebrafish larva.
- 25-26. (Canceled)

27. (Original) The method of claim 20, wherein the test agent is administered to the culture media of the zebrafish.
28. (Original) The method of claim 20, wherein the test agent is injected into the zebrafish.
29. (Canceled)
30. (Original) The method of claim 20, wherein the method is performed in an array format.
31. (Original) The method of claim 20, further comprising contacting the zebrafish with a dye.
32. (Canceled)
33. (Original) The method of claim 20, wherein the test agent is evaluated in combination with a second test agent.
34. (Original) The method of claim 20, wherein the test agent is a small molecule used or being considered for use as a pharmaceutical agent.
35. (Original) The method of claim 20, wherein the parameter of heart contractility is measured by recording the zebrafish heartbeat and analyzing the recording.
36. (Currently amended) The method of claim 20, wherein the recording as a video recording, and the parameter of heart contractility is measured by determining an average pixel intensity or density of the video recording throughout a specified region of the heart for a given time interval, and measuring the time between peaks of the intensity or density.
- 37-39. (Canceled)

40. (Currently amended) A method of evaluating the effect of a plurality of compounds on a parameter of heart contractility in a mammal, the method comprising:

contacting a developing zebrafish heart with a plurality of compounds,  
obtaining a recording of the zebrafish heart,  
evaluating a parameter of heart contractility in the zebrafish by analyzing the recording automatically using a computer program; and  
correlating the effect of the plurality of compounds on the parameter of heart function in the zebrafish heart with a predicted effect on a mammalian heart.

41. (Original) The method of claim 40, wherein the parameter is heart rate or QT-interval.

42. (Currently amended) ~~The method of claim 40;~~ A method of evaluating the effect of a plurality of compounds on a parameter of heart contractility in a mammal, the method comprising:

contacting a developing zebrafish heart with a plurality of compounds;  
evaluating a parameter of heart contractility in the zebrafish; and  
correlating the effect of the plurality of compounds on the parameter of heart function in the zebrafish heart with a predicted effect on a mammalian heart, wherein the parameter is ejection fraction, repolarization, or conduction velocity.

43. (Original) The method of claim 40, wherein the plurality of compounds is contacted simultaneously.

44. (Original) The method of claim 40, wherein the plurality of compounds is contacted separately.

45. (Original) The method of claim 40, wherein one of the plurality of compounds is a hormone.

46. (Canceled)

47. (Original) The method of claim 40, wherein the test agent is a small molecule.

48. (Original) The method of claim 40, wherein the parameter of heart contractility is measured by recording the zebrafish heartbeat and analyzing the recording.

49. (Currently amended) The method of claim 40, wherein the recording as a video recording, and the parameter of heart contractility is measured by determining an average pixel intensity or density of the video recording throughout a specified region of the heart for a given time interval, and measuring the time between peaks of the intensity or density.

50. (Original) The method of claim 40, wherein the parameter of heart contractility is measured by performing an EKG on the zebrafish.

51. (Canceled)

52. (Currently amended) A method of evaluating the effect of a plurality of different treatments, the method comprising:

(a) providing an array of a plurality of individual regions, wells or addresses, each region, well or address of the plurality comprising a zebrafish larva being provided with a test treatment that differs from those at other regions, wells or addresses of the plurality;

(b) obtaining a plurality of recordings of the zebrafish at the plurality of regions, wells, or addresses, and

~~(b)~~ (c) evaluating a parameter of heart contractility of the zebrafish at each of the plurality of regions, wells or addresses by analyzing each of the plurality of recordings automatically using a computer program, thereby evaluating the effect of a plurality of different treatments.

53. (Original) he method of claim 52, wherein the plurality of different treatments comprises a plurality of different compounds.

54. (Original) The method of claim 52, wherein the plurality of different treatments comprises a compound at a plurality of different concentrations or dosages.

55. (Original) The method of claim 52, wherein the plurality of different treatments comprises a first compound in combination with a plurality of different second compounds.

56. (Canceled)

57. (Original) The method of claim 52, wherein the parameter of heart contractility is measured by recording the heartbeat of the plurality of zebrafish and analyzing the recording.

58. (Currently amended) The method of claim 52 wherein the recordings are video recordings, and the parameter of heart contractility is measured by determining an average pixel intensity or density of each of the video recordings throughout a specified region of the heart of each of the plurality of zebrafish for a given time interval, and measuring the time between peaks of the intensity or density.

59. (Canceled)

60. (Original) The method of claims 53, wherein the plurality of different compounds is a plurality of different small molecules.

61-70. (Canceled)

71. (New) The method of claim 16, wherein permeabilizing the zebrafish comprises a step of enzymatic digestion.

72. (New) The method of claim 32, wherein permeabilizing the zebrafish comprises a step of enzymatic digestion.

73. (New) An nucleic acid comprising the sequence of SEQ ID NO:2.

74. (New) A zebrafish comprising an oligonucleotide that inhibits expression of KCNH2.

75. (New) The zebrafish of claim 74, wherein the oligonucleotide comprises the sequence of SEQ ID NO:2.

76. (New) A method of decreasing expression of KCNH2 in a zebrafish comprising injecting a zebrafish with an oligonucleotide that inhibits expression of KCNH2.

77. (New) The method of claim 76, wherein the zebrafish is an embryo.

78. (New) The method of claim 76, wherein the oligonucleotide comprises the sequence of SEQ ID NO:2.

79 (New) A transgenic zebrafish comprising a cell which comprises a cardiac myosin promoter sequence operably linked to a heterologous coding sequence.



80. (New) The zebrafish of claim 79, wherein the cardiac myosin promoter is a cardiac myosin heavy chain promoter.

81. (New) The zebrafish of claim 79, wherein the cardiac myosin promoter is a cardiac myosin light chain promoter.

82. (New) The zebrafish of claim 81, wherein the cardiac myosin light chain promoter comprises the sequence of SEQ ID NO:1.

83. (New) A method of evaluating a test agent for cardiototoxicity in a mammal, the method comprising:

- contacting a zebrafish heart with a test agent and a voltage sensitive dye;
- evaluating the QT interval of the zebrafish heart using the voltage sensitive dye; and
- identifying a test agent that causes an abnormality in QT interval in the zebrafish heart as a cardiotoxic agent in a mammal.